Chapter 1

Introduction
1.1 Urolithiasis

Urolithiasis (from Greek oûron, "urine", + lithos, "stone", + -iásis) is the formation of urinary calculi or urinary stones, which are formed or located anywhere in the urinary system (Pearle et al., 2007). It comprises of nephrolithiasis (the formation of kidney stones), ureterolithiasis (the formation of stones in the ureters) and cystolithiasis (the formation of bladder stones) (Schwartz and Stoller, 2000). Stone formation in kidney is one of the oldest and the most widespread medical problem known to humans. Urinary calculi have been found in Egyptian mummies dating back to 4000 BC and in the remains of 1500-1000 BC old North American Indians. Reference to stone formation is also made in early Sanskrit documents from India between 3000 and 2000 BC (Patankar et al., 2008). Urolithiasis is a recurrent renal disease which affects 4% - 8% of the population in UK, 15% in US, 20% in the Gulf countries and 11% of the population in India. The disease affects all age groups from less than 1 year old to more than 70, with a male to female ratio of 2:1 (Ajayi et al., 2007; Chauhan et al., 2009).

Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate (Daudon et al., 1993). Calcium oxalate crystals, primary constituent of human renal stones, exist in the form of calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) (Bensatal and Ouahrani, 2008). The stone formation requires supersaturated urine which depends on urinary pH, ionic strength, concentration of calcium, phosphate, oxalate, sodium and uric acid ions and complexations. Various substances in the body have an effect on one or more of the above processes, thereby influencing a person’s ability to promote or prevent stone formation (Basavaraj ., et al 2007). Hydroxyapatite, Struvite, Brushite, Uric acid stone are also found in urinary system (Pathak et al., 2010). The whole urinary system with kidney stone location is shown in Fig. 1.
Type of kidney stones

Calcium-containing stones

The most common type of kidney stones worldwide contains calcium. In fact, calcium-containing stones represent about 80% of all cases. They typically contain calcium oxalate either alone or in combination with calcium phosphate in the form of apatite or brushite (Coe et al., 2005). In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage (Shah et al., 2015).

Struvite stones

About 10-15% of urinary calculi are composed of struvite (ammonium magnesium phosphate, $\text{NH}_4\text{MgPO}_4\cdot6\text{H}_2\text{O})$. Struvite stone is (also known as "infection stones", urease or triple-phosphate stones) most often in the presence of infection by urea-splitting bacteria (Vijaya et al., 2013).
Uric acid stones

About 5-10% of all stones are formed from uric acid. People with certain metabolic abnormalities, including obesity may produce uric acid stones. They also may form in association with conditions that cause hyperuricosuria (an excessive amount of uric acid in the urine) with or without hyperuricemia (an excessive amount of uric acid in the serum). They may also form in association with disorders of acid/base metabolism where the urine is excessively acidic (low pH) (Moe, 2006; Johri et al., 2010).

Mechanism of stone formation

The development of kidney stone is determined by two key processes, crystal formation and crystal retention. Changing condition in ion composition, pH, osmolarity and volume of the intratubular fluid may frequently challenge the solubility of the urinary calcium salts, calcium oxalate and calcium phosphate and crystals may form in the tubular fluid as a result of supersaturation (Kok, 1996; Evan et al., 2008).

In the literature the question frequently arises whether crystal adhesion either is the consequence or the cause of the phenotypical alterations associated with epithelial injury and repair. The fact that in vitro adhesion of crystals particularly occurs at injured/regenerating epithelia and not at normal cells suggests that a dedifferentiated phenotype causes crystal adhesion (Bigelow et al., 1998; Verkoelen et al., 1998). However, crystals (and/or concomitant high concentrations of calcium, oxalate or phosphate) have been found to induce injury, proliferation, production of inflammatory mediators and oxidative stress upon contact with epithelial cells in vitro, suggesting that epithelial dedifferentiation is a consequence rather than a cause of crystal adhesion (Lieske et al., 1992; Aihara et al., 2003; Umekawa et al., 2003).

In fact, three types of crystal-cell interactions occur (Fig.2): (1) crystal molecules hold to cell-surface which are expressed by injured/regenerating cells, (2) weak
interactions as expected during crystals passing with the tubular fluid flow shortly get in contact with the tubular wall and (3) crystal-cell contact as expected during obstruction of the tubular lumen. As crystals do not hold fast to normal epithelial cells, the crystal adhesion is the primary cause of cellular injury and phenotypical alterations. Rather, forced contacts and passing interactions, which may occur to normal epithelia cell, are to be considered as crystal-cell interactions and inducing initial injury. Although the exact mechanical and chemotoxic nature of crystal-induced epithelial injury remains to be determined, non-adhesive interactions may direct the normal epithelium towards a crystal-binding phenotype in such a way that a crystal may induce its own adhesion or that of subsequently passing crystals (Kumar *et al.*, 1991; Xue *et al.*, 2009).

**Fig. 2** Schematic overview of urological crystal-cell interactions in the kidney
Medical Therapy

There are different treatment options for different stone types. Now there is convincing evidence that by treating specific biochemical abnormalities, the recurrence rate can be reduced (Alok et al., 2013). The list of synthetic drugs used in the treatment of stone diseases is given in Table 1.

Table 1. List of synthetic drugs used in the treatment of stone diseases.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drugs</th>
<th>Category</th>
<th>Mechanism of action</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amiloride</td>
<td>Diuretic</td>
<td>Na⁺ reabsorption in late distal tubule and collecting duct</td>
<td>Kidney diseases</td>
</tr>
<tr>
<td></td>
<td>(Midamor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Allopurinol</td>
<td>Analogue of hypoxanthine</td>
<td>It inhibits xanthine oxidase and prevent the synthesis of urate.</td>
<td>Urinary infections, Calculi</td>
</tr>
<tr>
<td>3</td>
<td>Cholestyramine</td>
<td>Bile-acid sequestrates</td>
<td>Increases in hepatic LDL receptors.</td>
<td>Kidney diseases</td>
</tr>
<tr>
<td>4</td>
<td>Cholic acid</td>
<td>Bile acid derivatives</td>
<td>It induces bile flow</td>
<td>Gall stone diseases</td>
</tr>
<tr>
<td>5</td>
<td>Digoxin</td>
<td>Cardiac glycoside</td>
<td>Inhibition of Na⁺, K⁺, ATPase</td>
<td>Kidney diseases</td>
</tr>
<tr>
<td>6</td>
<td>Etidronate</td>
<td>Bisphosphate</td>
<td>It prevent hydroxyl apatite dissolution</td>
<td>Kidney stone</td>
</tr>
<tr>
<td>7</td>
<td>Fluvastatin</td>
<td>Statin</td>
<td>Reduction of LDL levels.</td>
<td>Gall bladder diseases</td>
</tr>
</tbody>
</table>

Surgical Treatment

Surgery is as an option for cases where other approaches have failed or should not be tried. Surgery may be needed to remove a kidney stone, if it does not pass after a reasonable period of time and causes constant pain, is too large to pass on its own or is trapped in a difficult place, blocks the flow of urine, causes ongoing urinary tract infection, damages kidney tissue or causes constant bleeding, has grown larger.
Extracorporeal Shockwave Lithotripsy

Extracorporeal shockwave lithotripsy (ESWL) (Fig. 3) is the most frequently used procedure for the treatment of kidney stones. In ESWL, shock waves that are created outside the body travel through the skin and body tissues until they hit the denser stones. The stones break down into sand like particles and are easily passed through the urinary tract in the urine.

In ESWL treatment, recovery time is short and most people can resume normal activities in few days. Complications may occur with ESWL. Most patients have blood in their urine for a few days after treatment. Bruising and minor discomfort in the back or abdomen from the shock waves are also common. Another complication may occur if the shattered stone particles cause discomfort as they pass through the urinary tract. Sometimes the stone is not completely shattered with one treatment and additional treatments may be needed (Chaussy et al., 1984; McAteer and Evan, 2008).

Fig. 3 Photographic image of extracorporeal shockwave lithotripsy
Percutaneous Nephrolithotomy

Sometimes percutaneous nephrolithotomy is recommended to remove a stone. Photographic image of percutaneous nephrolithotomy is shown in Fig. 4. This treatment is often used when the stone is quite large or in a location that does not allow effective use of ESWL. In this procedure, the surgeon makes a tiny incision in the back and creates a tunnel directly into the kidney. Using a nephroscope, the surgeon locates and removes the stone. For large stones, some type of energy probe (ultrasonic or electrohydraulic) may be needed to break the stone into small pieces. Generally, patients stay in the hospital for several days and may have a small tube called a nephrostomy tube left in the kidney during the healing process. One advantage of percutaneous nephrolithotomy over ESWL is that the surgeon removes the stone fragments instead of relying on their natural passage from the kidney (Michel et al., 2007).

Fig. 4 Photographic image of percutaneous nephrolithotomy

Ureteroscopic Stone Removal

Ureteroscopy may be needed for mid and lower ureter stones. No incision is made in this procedure. Instead, the surgeon passes a small fiber-optic instrument called an ureteroscope through the urethra and bladder into the ureter. The surgeon then locates the stone and either removes it with a cage-like device or shatters it with a special instrument.
that produces a form of shock wave. A small tube or stent may be left in the ureter for few days to help the lining of the ureter to heal. Before fiber optics made ureteroscopy possible, physicians used a similar "blindbasket" extraction method. Photographic image of ureteroscopic stone removal is show in Fig. 5. But this outdated technique should not be used because it may damage the ureters (Delvecchio et al., 2000).

**Fig. 5** Photographic image of ureteroscopic stone removal

### 1.2 Oxidative stress and urolithiasis

Free radicals are unstable chemical species with one or more unpaired electrons in the outer orbit which makes them highly reactive. While enzymatic and nonenzymatic systems preserve the antioxidant/oxidant status, the defense system becomes overwhelmed during oxidative stress, a metabolic derangement due to an imbalance caused by excessive generation of reactive oxygen species (ROS) or a diminished antioxidant capacity. Free radicals generated by a wide variety of processes are involved in various diseases (Floyd, 1990; Baker et al., 1995). Free radicals damage lipids, proteins and DNA with consequent effects ranging from cell dysfunction to neoplasia. Lipid peroxidation represents oxidative tissue damage by superoxide, hydroxyl radicals
and hydrogen peroxide, which results in structural alteration to membranes and the functional impairment of the cellular components. With respect to oxidative enzymes xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH) dependent oxidase have been reported as potentially important sources of oxygen radicals in mesangial and glomerular epithelial cells (Neale et al., 1993; Jones et al., 1995).

ROS play a key role in the pathophysiological processes of a wide range of renal diseases. Oxidative stress may alter the structure and function of the glomerulus because of the effect of ROS on mesangial and endothelial cells (Klahr, 2001). Renal cellular exposure to oxalate or calcium oxalate crystals leads to the production of ROS, development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appear to play a significant role in stone formation.

Oxidative stress mediates a wide range of renal impairments, ranging from acute renal failure (Shah, 2001), obstructive nephropathy (Klahr, 2001), hyperlipidemia (Sakatsume et al., 2001) and glomerular damage (Kitamura and Ishikawa, 1999) to chronic renal failure and hemodialysis and associated inflammation (Handelman et al., 2001). Thus, increased levels of malondialdehyde and F$_2$-isoprostanes, two products of lipid peroxidation, have been reported in various clinical settings associated with renal damage (Martin-Mateo et al., 1999). Kidney stone formation decreases the super oxide dismutase (SOD), catalase, glutathione peroxidase (GPx), gluthione reduced (GSH) in serum. Reduction and total elimination of crystal deposition is associated with restoration of anti-oxidation defense of the kidney by increasing activities of the above stated enzyme.

The naturally occurring antioxidants, such as fruits, vegetables, tea, medicinal plants would attenuate the renal damage caused by oxidative challenges. Polyphenolic compounds could play a major role in enhancing the antioxidant system, since they behave as ROS scavengers, metal chelators and enzyme modulators (Pietta et al., 1998).
Challenges and future aspects of medicinal plants

In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. Plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects (Prasad et al., 2007).

Today medicinal plants are very important for the development of new drugs. People are using herbal drugs because of its safety, efficacy and lesser side effects. Plants and plant products have been utilized with varying success to cure and prevent diseases. At present demand of natural plants derived products are increasing day by day globally (Sharma et al., 2008).

Plants provide a cheapest source of medicine for majority of world population, which are considered as quite safe, with minimal or no side effects (Bashir and Gilani, 2009). It has been estimated that about 75,000 species of higher plants exist on the earth. A reasonable estimate of about 10% has been used in traditional medicine. However, perhaps only about 1% of these are acknowledged through scientific studies to have therapeutic value when used in extract form by human (Ningthoujam et al., 2012).

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is strong and can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products (Clark,1996).
It is worthwhile to look for an alternative option by using medicinal plants or phytotherapy for kidney stone formation. A number of plant drugs have been used in many parts of the world for the treatment of urolithiasis (Grases et al., 1995; Viel et al., 1999; Laroubi et al., 2007). Numerous herbs, such as *Ammi visnaga* (Khan et al., 2001), *Bergenia ligulata* (Bashir and Gilani, 2009), *Cynodon dactylon* (Atmani et al., 2009), *Herniaria hirsuta* (Atmani et al., 2004), *Nigella sativa* (Hadjzadeh et al., 2007), *Phyllanthus niruri* (Freitas et al., 2002), *Sesbania grandiflora* (Doddola et al., 2008), *Trigonella foenum-graecum* (Laroubi et al., 2007), *Launaea procumbens* (Makasana et al., 2014), *Helichrysum graveolens* and *Helichrysum stoechas* (Orhan et al., 2015), pomegranate (Tugcu et al., 2008), lemon tea (Touhami et al., 2007) and green tea (Itoh et al., 2005) have been reported to show an antiurolithiatic effect.

### 1.3 Selection of the plant for present study

Generally selection of the plant is done by 4 methods i) random choice of plant species, ii) choice based on ethno medical use, iii) follow up of existing literature on the use of the species and iv) chemotaxonomic approaches. Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Therefore, based on the first and third approach selection of the plant *Terminalia arjuna* Wight & Arn. had been made in the present work.

*Terminalia arjuna* Wight & Arn. is distributed throughout the greater part of India. The plant *Terminalia arjuna* (TA) has a long history of medicinal uses primarily in the preparation of Ayurvedic formulations (Nammi et al., 2003). Leaf has been reported for anti cancer activity (Kandil and Nassar, 1998) antifungal and antibacterial activity (Tripathi and Singh, 1996), antioxidant activity (Kuvad et al., 2014; Rakholiya et al., 2015). The bark of plant also possesses hypocholesterolic effects (Ram et al., 1997), cardio protective (Asha and Taju, 2012), antiherpes simplex virus type 2 (Cheng et al., 2002), cardiovascular disorders (Dwivedi, 2007), coronary risk factors (Dwivedi et al., 2000), myocardial necrosis (Sumitra et al., 2001), polyphenols that includes flavonols, flavones and phenyl propanoids are also useful in curing cancer (Dwivedi and Udupa,
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1989). Antidyslipidemic and antioxidant activities of different fractions of T. arjuna stem, bark is reported by Chander et al., (2004). Morshed et al., (2011) reported antimicrobial and cytotoxicity activity of T. arjuna 50 % ethanol extract of bark; anti-inflammatory, immunomodulatory and antinociceptive activity of T. arjuna bark powder is reported by Halder et al., (2009) and antioxidant and antidiabetic property by Raghavan and Kumari, (2006). Wound healing activity of phytoconstituents of T. arjuna is reported by Chaudhari and Mengi (2006). Ulcer protective effect and antimutagenic effect is reported by Devi et al. (2007) and Kaur et al., (2002) respectively, hepatoprotective effect by Subasini et al., (2007) and Reddy et al. (2008) reported the effect of T. arjuna extract on adriamycin induced DNA damage. Phytochemical investigations of the plant revealed the presence of many bioactive compounds like Arjunolic acid (King et al., 1954), Arjunic acid and Arjunoglycoside (Jain et al., 2009), Ellagic acid (Kandil and Narsar, 1998), Terminolitin (Tripathi and Singh, 1996), Arjunetoside (Pawar and Bhutani, 2005). However, so far no scientific study has been reported regarding the antiurolithiatic property of the T. arjuna. Therefore in the present study, an attempt has been made to evaluate the antioxidant and antiurolithiatic efficacy of T. arjuna leaf, stem and bark.

The objectives of the present study are as mentioned below:

- Review of literature:
  - Reported antioxidant activity of some medicinal plants
  - Reported antiurolithiatic activity of some medicinal plants

- Pharmacognostic study of T. arjuna leaf, stem and bark
- Phytochemical and Physiochemical analysis of T. arjuna leaf, stem and bark
- Fractionation of the best solvent extract of T. arjuna leaf, stem and bark
- In vitro antioxidant activity of T. arjuna leaf, stem, bark and fractions
- In vitro antiurolithiatic activity of T. arjuna leaf, stem and bark
- In vivo antiurolithiastic activity of T. arjuna bark
1.4 References


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